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INVESTIGATION OF ENDOPHYTIC FUNGI *Trichoderma* sp. IN FORM OF POWDER AND DIFFERENT DOSES AGAINST ROOT-KNOT NEMATODE *Meloidogyne* spp.

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Abstract: *The term "endophytes" includes a suite of microorganisms that grow intra-and/or intercelullarly in the tissues of higher plants without causing over symptoms on the plants in which they live, and have proven to be rich sources of bioactive natural products. Root-knot nematodes Meloidogyne spp are serious pests of many cultivated crops around the word and is estimated economic losses around US $ 100 billion annually. A Greenhouse experiment was conducted in five treatment (Doses) of endophytic fungi Trichoderma sp namely T0 (control), T1 (Trichoderma with 200 mg/kg media), T2 (Trichoderma with 500 mg/kg media), T3 (Trichoderma with 1000 mg/kg media) and T4 (Trichoderma with 1500 mg/kg). All of the treatment doses of endophytic fungi Trichoderma sp. in term of intensity damaged and population density of meloidogyne-egg and meloidogyne-J2 statistically different to control.*

Keywords: *Endophytic fungi, Trichoderma sp, Root-knot nematode, Meloidogyne spp.*

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an eudicotiledonous plant that belongs to the family Solanaceae together with other economically important crops such as pepper, eggplant and potato. It is the most important grown fresh market vegetable world wide with more than 5 million hectares harvested in China, United States of America, India, Turkey and Egypt as the five first producers, respectively. Tomato is a good source of vitamins A and C, potassium and fiber. It is rich in lycopene (Di Mascio *et al*, 1989), which is used in the fight against cancer, especially the prostate cancer (Giovanucci *et al*, 1995).

Plant parasitic nematodes are responsible for over $ 100 billion dollars in economic losses worldwide to a variety of crops. Root-knot nematodes are the most economically important group of plant parasitic nematodes worldwide, reducing both yield and crop quality (Sasser and Freckman, 1987; Moens *et al*., 2009). Infected plants show reduced growth, swollen roots which develop into the typical root-knot galls, are two, or three times larger in diameter as healthy root. Root-knot nematodes are very difficult to control because they are polyphagous, where its over 2000 plants species is a highly specialized and complex feeding relationship with their host (Hussey and Janssen, 2002). The life cycle is almost completely confined inside the host plant and high reproductive capacity. Although chemical control is still a common method for reducing nematode population, there is a considerable public pressure to limit or even ban the use of nematicides. Many nematicides are highly toxic and sometimes very mobile in the soil because of their solubility in water. Concern over these chemicals has led to an increased interest in biological control in order to achieve more environmentally friendly methods of reducing nematode damage.

The term of endophyte was coined by the German scientist, Heinrich Anton De Bary in 1884, and used to define fungi and bacteria occurring inside plant tissues without causing any apparent symptoms in the host (Schulz *et al*, 2006). In the last few years endophytic fungi have been detected in hundreds of plants including such important agricultural commodities as bananas (Nur Amin, 1994), maize (Nur Amin, 2013a); tree palm oil (Nur Amin *et al*., 2008) and cocoa plant (Nur Amin *et al*., 2014). A number of authors have documented that the presence of endophytic fungi provide a protection of the plant hosts against insect herbivore (Clement *et al*, 2005), parasitic nematodes (Nur Amin, 1994; 2013b; Elmi *et al*, 2000), and plant pathogens (Dingle and Mc Gee, 2003; Wicklow *et al*, 2005).

Endophytic fungi *Trichoderma* sp. have been isolated from different host plant such as maize (Nur Amin, 2013a), cocoa (Nur Amin *et al*., 2014), medicinal plant of Makassar fruit (Nur Amin *et al*., 2015). The purpose of the present investigation was to see of the eficacy of endophytic fungi *Trichoderma* sp. in different doses towards root-knot nematode *Meloidogyne* spp. in greenhouse

MATERIAL AND METHODS

1. Source of Endophytic Fungi *Trichoderma* sp.

Endophytic fungi *Trichoderma* sp. was originally isolated from cortical tissue of surface sterilized root of tomato plant *Solanum lycopersicum.*

2. Source of *Meloidogyne* spp.

The root-knot nematode *Meloidogyne* spp. was originally isolated from an infested field on tomato plant in distric Barombong, south Sulawesi, Indonesia. The extraction to obtained *Meloidogyne*-J2 by using the modified extraction technique of Hooper *et al* (2005). Roots were washed free from soil under tap water, cut into 1 cm pieces and macerated in a warring blender at high speed for 20 s and collected in a glass bottle. Sodium hypochloride (NaOCl) was added to obtain a final concentration of 1.5% active Chlorine and the bottle was shaken vigorously for 3 min. The suspension was then thoroughly washed with tap water through a sieve combination 250, 100, 45 and 25 µm mesh to remove the NaOCl. Eggs were collected on the 25 µm sieve and then transferred to a glass bottle. The egg suspension was supplied with oxygen from an aquarium pump over 10 days to induce juvenile hatching. To separate active J2 from unhatched eggs or dead J2, a modified Baermann technique over 24 hours was used. The collected active J2 were adjusted to 1000 J2 5 ml-1 and used immediately as inoculum.

3. Investigation of Endophytic Fungi *Trichoderma* sp. In Form of Powder and Different Doses against Root-Knot Nematode *Meloidogyne* spp. In Greenhouse

Tomato seeds (*Lycopersicon esculentum*) Var. Marglobe were surface sterilized by first shaking them in a 75% Ethanol solution for 1 min and then in a 1.5% Sodium hypochloride (NaOCl) solution for 3 min. The seeds were washed with tap water and transferred to sterile sand for germination at greenhouse conditions.

The tomato seedlings were transplanted into 1000-ml polybag (20 x 30cm) containing a sterilized of a mixture of soil-sand-cow manure with the ratio (2:1:1). At the time of

transplanting a different doses of endophytic fungi *Trichoderma* sp. was applied as a soil drench around the stem where as the control was treated with 10 ml sterile aquadest. Simultaneously 1000 J2 of *Meloidogyne* spp. were added in 10 ml of tap water in three holes around the stem.

The investigation is based on the completely randomized design (CRD) with five treatment (Doses) of endophytic fungi *Trichoderma* sp namely T1 (control), T2 (Trichoderma with 200 mg/kg media), T3 (Trichoderma with 500 mg/kg media), T4 (Trichoderma with 1000 mg/kg media) and T5 (Trichoderma with 1500 mg/kg). Investigation consisted with 5 replications in which each treatment plant using 3 samples, that contained 75 units of the plant . The treatments used were :

T0 = Control (Tomato Plant + 1000 *Meloidogyne*-J2)

T1 = (Tomato Plant + 200 mg/kg media + 1000 *Meloidogyne*-J2)

T2 = (Tomato Plant + 500 mg/kg media + 1000 *Meloidogyne*-J2)

T3 = (Tomato Plant + 1000 mg/kg media + 1000 *Meloidogyne*-J2)

T4 = (Tomato Plant + 1500 mg/kg media + 1000 *Meloidogyne*-J2)

The investigation was terminated 21 days after nematode inoculation at which time intensity damaged, means of population meloidogyne-egg, meloidogyne-J2 on the root and in the soil were measured.

DATA ANALYSIS

ANOVA was also performed to determine the effects of doses endophytic fungi to intensity damaged, means of population meloidogyne-egg, meloidogyne-J2 on the root and in the soil. The percent data were arcsine-transformed before being subjected to ANOVA. When significant differences were detected, means were separated using Tukey's test at 5% probability level.

RESULT AND DISCUSSION

Significant differences in intensity damaged to all of the treatment doses of endophytic fungi to control were observed. The treatment of T2 (500 mg / kg media) and T3 (1000 mg / kg media) showed significant difference with all treatments (T1 = control; T2: 200 mg / kg of media and T5 = 1500 mg / kg media), respectively of 30% and 29% intensity damaged compared with control treatment 96% (T1), 49 % (T2) and 41 % (T5) (Figure 1).

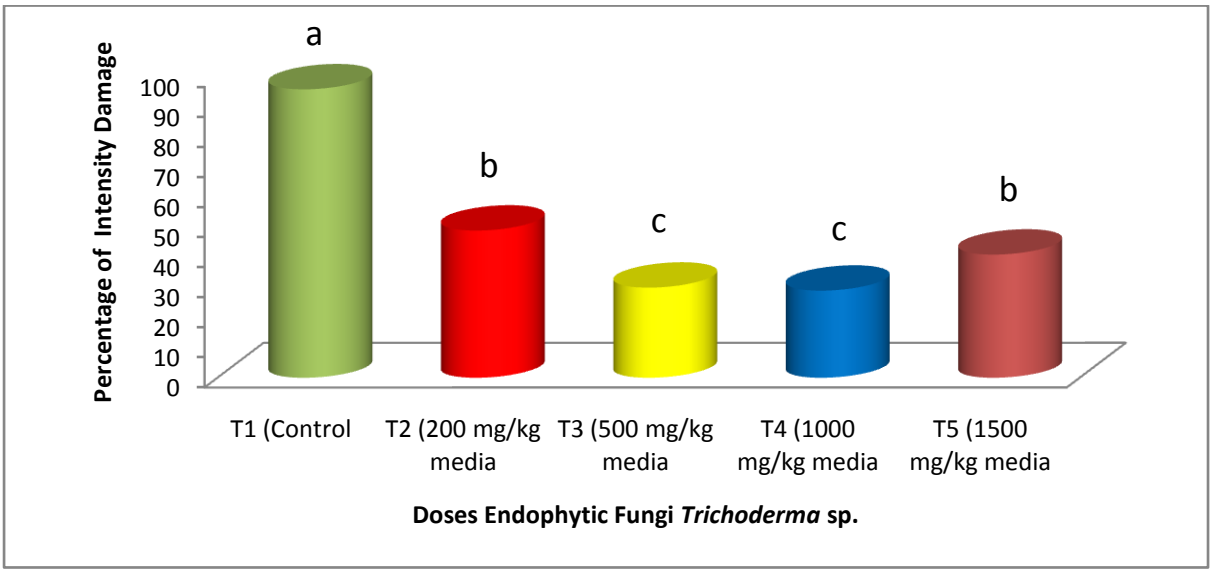

Figure 1. Percentage of Intensity Damage in different Doses in Form of Powder of Endophytic Fungi *Trichoderma* sp.

The population density of *Meloidogyne*-Egg on soil gram^{-100} and the root of tomato gram^{-1} in the treatment doses of endophytic fungi *Trichoderma* sp. were greatly reduced in all treatments over the control and significantly different. However, the *Meloidogyne*-egg population densities on the doses of 1000 mg/kg (T4) is the lowest to compare with other doses of endophytic fungi *Trichoderma* sp., by 427 on soil Gram^{-100} and 515 on root Gram^{-1} and significantly different wuth the other treatment (Table 1).

Table 1. Means of Population Density of *Meloidogyne*-Egg on Soil Gram^{-100} and the Root of Tomato Gram^{-1} in Different Doses in Form of Powder of Endophytic Fungi *Trichoderma* sp.

Concentration	Means of *Meloidogyne*-J2 on	
	Soil Gram^{-100}	Root of Tomato Gram^{-1}
T1	3097 a	2453 a
T2	2269 b	1598 b
T3	1415 c	954 c
T4	427 d	515 d
T5	1859 b	1058 c

Numbers in the same column followed by same letters are not significantly different (P=0.05, Tukey's test)

The population density of *Meloidogyne*-J2 on soil gram^{-100} and the root of tomato gram^{-1} in the treatment doses of endophytic fungi *Trichoderma* sp. were greatly reduced in all treatments over the control and significantly different. However, the *Meloidogyne*-J2 population densities on the doses of 1000 mg/kg (T4) is the lowest to compare with other doses of endophytic fungi *Trichoderma* sp., by 437 on soil Gram^{-100} and 831 on root Gram^{-1} (Table 2).

Table 2. Means of Population Density of *Meloidogyne*-J2 on Soil Gram^{-100} and the Root of Tomato Gram^{-1} in Different Doses in Form of Powder of Endophytic Fungi *Trichoderma* sp.

Concentration	Means of *Meloidogyne*-J2 on	
	Soil Gram^{-100}	Root of Tomato Gram^{-1}
T1	2077 a	3118 a
T2	1092 b	2350 b
T3	647 d	1516 c
T4	437 e	831 d
T5	1142 c	2074 b

Numbers in the same column followed by same letters are not significantly different (P=0.05, Tukey's test)

The intensity damaged and the population density of *Meloidogyne*-Egg and *Meloidogyne*-J2 reduced on the treatment doses of endophytic fungi *Trichoderma* sp. The actuality condition is may be related to nematode penetration, in this case due to the influence of fungal colonization. Many researchers have shown that the endophytic fungi can reduce nematode penetration in different crops (Hallmann and Sikora, 1994a, b; Pocasangre, 2000; Vu, 2005; Dababat, 2007). For instance, the mutualistic endophyte *Fusarium oxysporum* Fo162 reduced penetration of *Meloidogyne incognita* in tomato and *Radopholus similis* in banana by 28% to 41% respectively (Vu, 2005; Dababat, 2007). Many studies have elucidated that fungal endophytes may alter chemical or physical properties of the root exudates or interact with the plants to produce chemical or hormone complex compounds which repel or interfere with nematode attraction (Diez and Dusenbury, 1989; Dababat and Sikora, 2007).

The other mechanisms to reduced of intensity damaged and the population density is related to induced systemic resistance. Induced systemic resistance (ISR) is commonly defined as "a phenomenon where by resistance to infectious disease is systemically induced by localized infection or treatment with microbial components or products or by a diverse group of structurally unrelated organic and inorganic compounds. The activity of the inducing agents is not due to antimicrobial activity perse or their ability to be transformed into antimicrobial agents. However, antimicrobial agents can induce resistance, and they provide protection from the time of application until ISR is fully expressed".

CONCLUSION

Based on the results from this study, we conclude that the treatment of doses 1000 mg/kg media was the best result in form of percentage damage and population dencity and have the potential as biological control agent against root-knot nematode *Meloidogyne* spp. There is however, need to evaluate the efficacy of the this doses of endophytic fungi *Trichoderma* sp. under field conditions.

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